

Molecular Fingerprints Identify Historic Pear Trees in Two U.S. National Parks

N. Bassil, J. Postman and K. Hummer
U.S. Department of Agriculture
Agricultural Research Service
National Clonal Germplasm Repository
Corvallis, Oregon
USA

S. Dolan
U.S. Department of Interior
National Park Service
Seattle, Washington
USA

L. Lawliss
U.S. Department of Interior, National Park Service
Richmond, California
USA

Keywords: *Pyrus*, DNA, microsatellite, SSR

Abstract

The U.S. Department of Interior, National Park Service (NPS), has developed conservation plans for historical orchards within National Park boundaries. Cultivar identification of significant fruit trees is an important part of these plans. The U.S. Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository (NCGR), in Corvallis, Oregon, maintains a genebank of world pear germplasm and uses simple sequence repeat (SSR) markers to fingerprint cultivars. These two agencies are collaborating to identify historic pear trees in U.S. National Parks using SSR fingerprints. The San Juan Island National Historical Park (NHP) north of Seattle, Washington, contains several remnant orchards with large surviving European pear (*Pyrus communis* L.) trees planted in the late 19th to early 20th centuries. The John Muir National Historic Site (NHS) in Martinez, California, also contains many old pear trees. Young leaf samples were collected from 31 pear trees at San Juan NHP and John Muir NHS in May 2006 and sent to the USDA genebank in Oregon for analysis. DNA was extracted and microsatellite fragments were amplified using 11 SSR primer pairs. Several known pear cultivars were also included that were suspected to be identical to NPS pears or that were available from nurseries at the time these locations were settled. Four pear trees growing at the English Camp at San Juan NHP had SSR fingerprints identical to 'Pound Pear'. Four other San Juan NHP pears were found to be identical to 'Bartlett' and one was identical to 'White Doyenne'. Other pear trees at San Juan NHS were identical to each other but did not match any of the standards. Seven pear trees at John Muir NHS were indistinguishable from 'Bartlett', and two trees were identical to each other but we were unable to match them to the standards. About 20 pear trees remain in an orchard where John Muir was buried in 1915 and are now a part of the National Historic Site. A large tree growing by his gravesite was confirmed as 'Bartlett'. DNA fingerprinting using SSRs is a promising technology for rapid identification of historical fruit trees even when no fruit is present. As additional pear accessions at the USDA genebank become fingerprinted more unknown historical trees will be identifiable.

INTRODUCTION

Many United States National Parks include old homesteads or orchards. A survey completed in 1992 determined that one third of all National Park properties contain historically significant fruit or nut trees (Dolan, 2007). The U.S. Department of Interior, National Park Service (NPS) has developed conservation plans for historic fruit trees and orchards within National Park boundaries, and cultivar identification is an important part of these plans. Old pear trees at the John Muir National Historic Site (NHS) in California

and at the San Juan Island National Historic Park (NHP) in Washington were selected to test the application of DNA fingerprinting for identification of unknown cultivars. The U.S. Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon, manages a world collection of pear germplasm including hundreds of historic cultivars that are available as identity standards (Hummer, 1994; Postman et al., 2006). Microsatellite or simple sequence repeat (SSR) markers have been developed and mapped in pear (Yamamoto et al., 2002b). Additional SSR markers were recently developed at NCGR from GenBank pear sequences and many appear to be polymorphic (Bassil et al., 2005). Pear cultivars, even old trees with no fruit and growing under challenging environmental conditions, should be easily identified using microsatellite markers and a library of SSR fingerprints from known cultivars.

John Muir National Historic Site

The John Muir NHS in Martinez, California, is a 136 hectare park where the home of this famous American naturalist is preserved. Muir's property included several large orchards in the 1800s, and today the park includes more than 3 hectares of orchards (NPS, 2006a). About 20 pear trees remain close to where Muir was buried in 1915. A large tree growing by his gravesite, and possibly the oldest pear tree in the park is thought to be the cultivar 'Bartlett' that was planted about 1880.

San Juan Island National Historic Park

San Juan Island NHP is off the coast of Washington north of Seattle. During the early 1800s the English maintained a camp on the north end of the island and the Americans maintained a camp on the south end (NPS, 2006b). Several large European pear trees planted in the late 19th to early 20th centuries remain at the sites of these two camps. Large and still productive pear trees growing near the beach and in nearby fields at the English Camp were planted by the Scotsman James Crook upon the departure of the British Royal Marines from the camp in 1872. Other pear trees are also growing nearby on land where Isaac Sandwith established a homestead in the 1880s. Some of the trees remaining in these locations were tentatively identified as 'Vermont Beauty', 'Pound', 'Bartlett' and 'Bosc' based on fruit characteristics and ripening seasons.

MATERIALS AND METHODS

In May 2006, NPS personnel collected young leaves from 21 old pear trees growing at San Juan Island NHP and from 10 trees growing at John Muir NHS on a plot that includes the grave of John Muir and several of his family members. Leaf samples were sent to the USDA genebank in Oregon for analysis. DNA was extracted from NPS leaf samples and also from leaves of 9 known cultivars growing at NCGR Corvallis using a modified Gentra PureGene protocol (Gentra Systems, Inc, Minneapolis, MN). The NCGR cultivars included were 'Bartlett', 'Beurre Bosc', 'Doyenne du Comice', 'Doyenne de Juillet', 'Pound', 'Vermont Beauty', 'Vicar of Winkfield', 'White Doyenne' and 'Winter Nelis'. These standard cultivars were chosen based on the presumed identity of NPS pears or because they were cultivars known to be available from nurseries at the time the park locations were settled. PureGene modifications included: addition of PVP (polyvinylpyrrolidone, MW = 40,000) to the lysis buffer; RNase A and Proteinase K digestion; and an additional protein precipitation step. PCR reactions were carried out in a total of 10 μ l of 1X Biolase NH₄ reaction buffer, 2 mM MgCl₂, 200 μ M each of dATP, dCTP, dGTP and dTTP, 0.3 μ M each of fluorescently labeled forward and standard reverse primers, 0.25 units of Biolase Taq DNA polymerase (Bioline Inc., Randolph, MA) and 2.5 ng of template DNA. Optimum annealing temperature was used for DNA amplification for 35 cycles in an Eppendorf Gradient thermocycler (Eppendorf, Westbury, NY) or an MJ Research (Watertown, Mass.) Tetrad thermocycler programmed for a 40 s denaturation step at 94°C, a 40 s annealing step at the optimum annealing temperature of the primer pair, and a 40 s extension step at 72°C. A final extension at 72°C for 30 min was used to maximize non-templated adenosine addition to the 5' ends.

Fragment size estimation was determined (Table 1) after separating the PCR products by capillary electrophoresis using the Beckman CEQ 8000 genetic analyzer (Beckman Coulter, Inc., Fullerton, CA).

Eleven SSR primer pairs (Table 1) were chosen based on product polymorphism in European pear, presence on different linkage groups if mapped and the ability to fit in three multiplexes. PCR products in a multiplex were pooled after PCR and separated by capillary electrophoresis. SSR names that start with CH were isolated from apple (Liebhard et al., 2002). The NH and PYR suffixes indicate SSRs isolated from pear (Bassil et al., 2005; Yamamoto et al., 2002a). We report results obtained with eight out of 11 SSRs. PYC-008 was monomorphic, however missing data prevented analysis of genetic fingerprints obtained with the two SSRs (PYC-011 and PYC-009b).

PCR products amplified from eight SSR primer pairs were scored for presence or absence using a Perl script. The data was imported into PowerMarker (Liu and Muse, 2004) and used for neighbor joining cluster analysis (Fig. 1) based on the proportion of shared alleles distance.

RESULTS

Eight microsatellite markers amplified 38 PCR products (alleles) from the 9 standard pear cultivars and 31 trees growing at National Park sites. Cluster analysis identified seven sets of identical trees or synonyms (Fig. 1). Seven pear trees growing at John Muir NHS and four trees at San Juan Island NHP were identical to 'Bartlett' (NCGR clone 38.001) based on eight polymorphic microsatellite loci. Four old trees in the English Camp at San Juan NHP were identified as 'Pound' pear, and a tree growing in the Sandwith Orchard at San Juan NHP was identified as 'White Doyenne' (Table 2). Nine other trees at San Juan NHP were found to be exact matches with other trees at the park, and two trees from John Muir NHS were identical to each other, but the identities did not match any of the known cultivars included in the study (Table 2).

DISCUSSION

Previous examination of pear fruits and trees at San Juan NHS and comparison to descriptions and images of cultivars from the appropriate era tentatively identified several trees as 'Vermont Beauty' and 'Winter Nelis'. However, none of the SSR fingerprints matched these cultivars. Several other trees at San Juan NHP were tentatively identified by visual examination as 'Belle Angevine' (synonym = 'Pound') and these identities were confirmed by the SSR fingerprints. Many old pear trees in historic cultural landscapes do not produce fruit due to lack of pollinizers or poor growing conditions, and as a result are very difficult to identify visually. SSR marker analysis recognized 27 of the 31 National Park pear trees as either identical to known cultivars or identical to other trees sampled. Even if the identity of two cultivars is not known, the synonymy based on SSR analysis confirms that they were clonally propagated cultivars and not seedling trees. As additional SSR fingerprints from known pear cultivars at the NCGR genebank are added to a searchable database, matching unknown and historic pear trees with their true identities will become possible. Historic orchards and fruit trees in U.S. National Parks not only provide rare views of orchard designs and tree management styles from earlier centuries, but like the USDA genebank they are also unique repositories of historic and unusual fruit cultivars. An historic fruit tree preserved at a National Park with a fingerprint that does not match any cultivar preserved in a USDA genebank provides a valuable opportunity to recapture a lost cultivar and the valuable genes it may contain. DNA fingerprinting using SSRs is a very promising technology for rapid identification of historic fruit trees. The usefulness of this technique is multiplied in the context of a partnership between an agency charged with conservation of agricultural resources and an agency charged with conservation of natural and cultural resources.

Literature Cited

Bassil, N.V., Postman, J.D. and Neou, C. 2005. *Pyrus* microsatellite markers from

- GenBank sequences. *Acta Hort.* 671:289–292.
- Dolan, S.A. 2007. A Fruitful Legacy: A historic context of fruit trees and orchards in the United States, from 1600 to the present. U.S. Department of Interior, National Park Service, Olmsted Center for Landscape Preservation, Pacific West Regional Office - Cultural Resources, and Park Historic Structures and Cultural Landscapes Program, draft.
- Hummer, K. 1994. Genetic resources of *Pyrus* and related genera at the Corvallis Repository. *Acta Hort.* 367:64–71.
- Liebhart, R., Gianfranceschi, L., Koller, B., Ryder, C.D., Tarchim, R., Weg, E.V.D. and Gessler, C. 2002. Development and characterisation of 140 new microsatellites in apple (*Malus x domestica* Borkh.). *Molecular Breeding* 10:217–241.
- Liu, K. and Muse, S. 2004. PowerMarker: new genetic data analysis software. Version 3.0. <http://www.powermarker.net>. Accessed 16 January, 2008.
- NPS. 2006a. John Muir National Historic Site, California. <http://www.nps.gov/jomu>. Accessed 1 March, 2007.
- NPS. 2006b. San Juan Island National Historic Park, Washington. <http://www.nps.gov/sajh>. Accessed 1 March, 2007.
- Postman, J., Hummer, K., Stover, E., Krueger, R., Forsline, P., Grauke, L.J., Zee, F., Ayala-Silva, T. and Irish, B. 2006. Fruit and nut genebanks in the US National Plant Germplasm System. *HortScience* 41(5):1188–1194.
- Yamamoto, T., Kimura, T., Shoda, M., Ban, Y., Hayashi, T. and Matsuta, N. 2002a. Development of microsatellite markers in the Japanese pear (*Pyrus pyrifolia* Nakai). *Molecular Ecology Notes* 2(1):14–16.
- Yamamoto, T., Kimura, T., Shoda, M., Imai, T., Saito, T., Sawamura, Y., Kotobuki, K., Hayashi, T. and Matsuta, N. 2002b. Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. *Theor. Appl. Genet.* 106:9–18.

Tables

Table 1. SSR primers used to differentiate pear cultivars at NCGR, Corvallis. Results were reported from highlighted primers in this study. Imp refers to imperfect GA motifs.

SSR*	Motif	T _a	Size Range	Linkage Group	Multiplex
PYC-011	(AG) ₇	50	109-115		1
NH007b	(AG) ₂₅	50	125-145	16	1
CH02c11	Imp	61	196-240	10	1
PYC-008	(TG) ₆	65	319		1
NH014a	(GA) ₁₇	55	68-82	17	2
CH03d02	Imp	61	171-232	11	2
PYC-010b	(TTTA) ₄ (TTA) ₆	65	331-338		2
PYC-013	(TC) ₁₂ TT(TC) ₈	62	99-105		3
CH04f06	Imp	64	154-186	14	3
CH02d12	Imp	62	219-241		3
PYC-009b	(CT) ₁₁	64	288-296		3

*CH primers from Liebhart et al. (2002); PYC primers from Bassil et al. (2005); NH primers from Yamamoto et al. (2002b).

T_a – temperature of annealing.

Table 2. Genetic fingerprints of pear trees from two U.S. National Park Historic Sites using eight microsatellite markers.

Tree Source	Primers								Pyr010b	CH02d12	CH04f06	Pyr013
	CH02c11	NH007b	CH03d02	NH014a	Pyr010b	CH02d12	CH04f06	Pyr013				
Bartlett NCGR 38.001	196 220 240	135 137	226 230	68 70	333 338	219 223	154 186	111 117				
John Muir NHS #78	196 220 240	135 137	226 230	68 70	333 338	219 223	154 186	111 117				
John Muir NHS #79	196 220 240	135 137	226 230	68 70	333 338	219 223	154 186	111 117				
John Muir NHS #85	196 220 240	135 137	226 230	68 70	333 338	219 223	154 186	111 117				
John Muir NHS #86	196 220 240	135 137	226 230	68 70	333 338	219 223	154 186	111 117				
John Muir NHS 33-1-1	196 220 240	135 137	226 230	68 70	333 338	219 223	154 186	111 117				
John Muir NHS 33-1-2	196 220 240	135 137	226 230	68 70	333 338	219 223	154 186	111 117				
San Juan NHP - English Camp E4	196 220 240	135 137	226 230	68 70	333 338	219 223	154 186	111 117				
San Juan NHP - English Camp E8	196 220 240	135 137	226 230	68 70	333 338	219 223	154 186	111 117				
San Juan NHP - English Camp E9	196 220 240	135 137	226 230	68 70	333 338	219 223	154 186	111 117				
San Juan NHP - Sandwith Orchard F1	196 220 240	135 137	226 230	68 70	333 338	219 223	154 186	111 117				
Pound NCGR 458.001	196 216 238	135 137	181	70 74	80 333 338	219 223	156 184	186 95 105 113				
San Juan NHP - English Camp E1	196 216 238	135 137	181	70 74	80 333 338	219 223	156 184	186 95 105 113				
San Juan NHP - English Camp E5	196 216 238	135 137	181	70 74	80 333 338	219 223	156 184	186 95 105 113				
San Juan NHP - English Camp E7	196 216 238	135 137	181	70 74	80 333 338	219 223	156 184	186 95 105 113				
San Juan NHP - English Camp E12	196 216 238	135 137	181	70 74	80 333 338	219 223	156 184	186 95 105 113				
White Doyenne NCGR 602.004	196 216 240	127 135	185 226	70 80	333 338	219 223	186	105 117				
San Juan NHP - Sandwith Orchard F3	196 216 240	127 135	185 226	70 80	333 338	219 223	186	105 117				
San Juan NHP - Sandwith Orchard F12	196 216 218	133 135	137 179 226	82	333 338	219 235	156 182	95 105 121				
San Juan NHP - Sandwith Orchard F13	196 216 218	133 135	137 179 226	82	333 338	219 235	156 182	95 105 121				
San Juan NHP - Sandwith Orchard F18	196 216 238	127 145	183 194	70 76	333 336 338	219 229 184	186	95 105				
San Juan NHP - Sandwith Orchard F19	196 216 238	127 145	183 194	70 76	333 336 338	219 229 184	186	95 105				
San Juan NHP - Sandwith Orchard F20	196 216 238	127 145	183 194	70 76	333 336 338	219 229 184	186	95 105				
San Juan NHP - English Camp E2	196 218 240	131 135	179 226	80	333 334 338	219 223 186		105 117				
San Juan NHP - English Camp E6	196 218 240	131 135	179 226	80	333 334 338	219 223 186		105 117				
San Juan NHP - English Camp E10	196 218 240	131 135	179 226	80	333 334 338	219 223 186		105 117				
San Juan NHP - Sandwith Orchard F2	196 218 240	131 135	179 226	80	333 334 338	219 223 186		105 117				
John Muir NHS B-11-1-1	196 218 228	135 137	179 226	76 80	333 338	228 235 176	186	113				
John Muir NHS B-11-1-3	196 218 228	135 137	179 226	76 80	333 338	228 235 176	186	113				
John Muir NHS 6F-1-18	196 216 240	125 137	181 189	70 78	333 338	219 238 156	186	105 113				
San Juan NHP - English Camp E3	196 240	137	175 226	68 70	333 338	219 236 154	182	99 117				
San Juan NHP - English Camp E11	196 226 238	135	171 210	70 80	333 338	223 241	154 186	99 113				
San Juan NHP - Sandwith Orchard F5	196 218 240	131 137	179 226	70 80	334 338	219 236 184	186	105 121				
Bosc NCGR 1165.001	196 218 240	135	181 226	70 76	333 336 338	219 223 184	186	105 117				
Doyenne du Comice NCGR 148.001	196 216 228	135	185 226	70 76	333 338	223 228 176	182	113 117				
Doyenne de Juillet NCGR 184.002	196 226 240	135	185 210	70 80	333 338	223 241	186	99 105				
Vermont Beauty NCGR 591.001	196 216 238	135	185 226	70 74	333 338	219 223 184	186	105 117				
Vicar of Winkfield NCGR 1172.001	196 216 230	135 137	226 232	70	331 338	219 228 156	182	105 117				
Winter Nellis NCGR 1164.001	196 228 240	135 137	179 185	70	333 338	223 236 156	184	113 121				

Figures

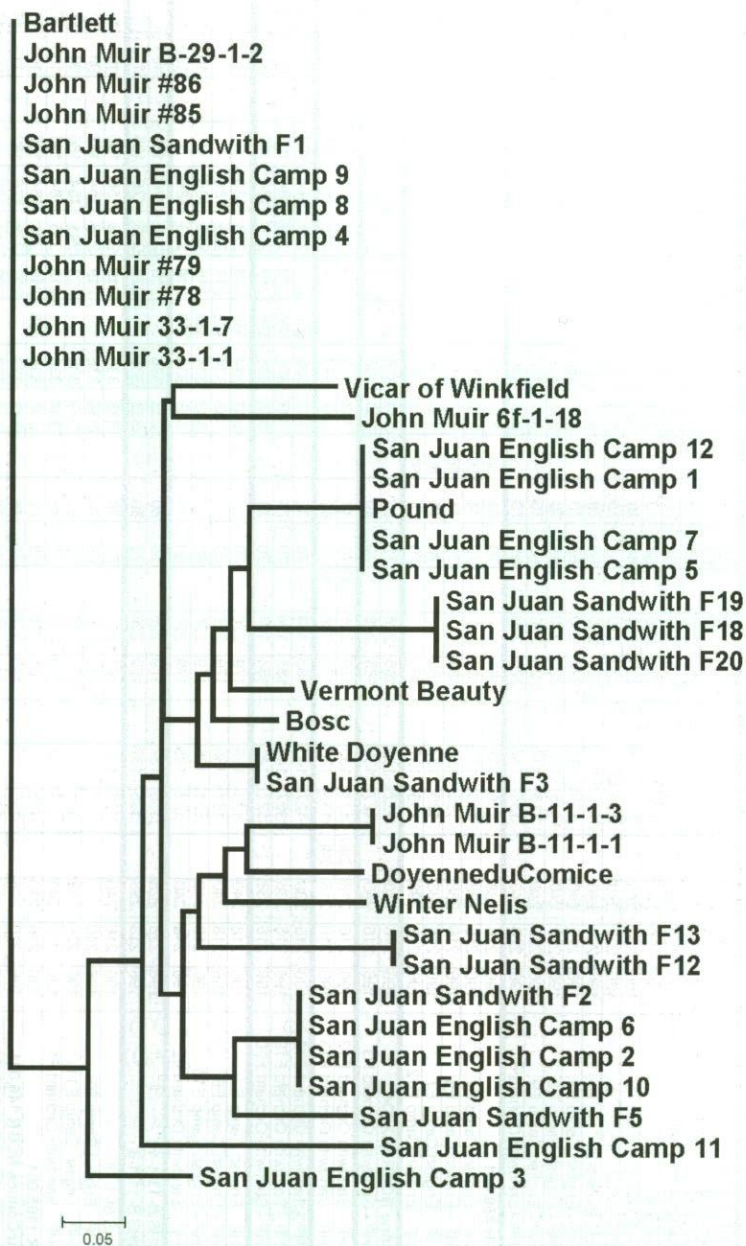


Fig. 1. Neighbour joining cluster analysis of 31 NPS pears and eight standard cultivars based on the proportion of shared alleles distance using eight SSR markers.